

# A $\beta$ -sheet peptide inhibitor of E47 dimerization and DNA binding

Indraneel Ghosh and Jean Chmielewski

**Background:** Many transcription factors are active only in their dimeric form, including the basic-helix-loop-helix (bHLH) family of transcription factors. The disruption of the dimer therefore presents a means of inhibiting the biological functions of such transcription factors. E47 is a homodimeric bHLH transcription factor with a four-helix bundle dimerization interface. Here, we investigate the concept of dimerization inhibition using peptides derived from the dimerization domain of E47.

**Results:** We have synthesized several peptides corresponding to the E47 dimerization interface that inhibit E47 DNA-binding activity with  $IC_{50}$  values in the range of 3.6–120 mM. Interestingly, helix II, a peptide corresponding to the carboxy-terminal helix of the E47 dimerization interface, adopted a  $\beta$ -sheet structure in solution, as shown using circular dichroism (CD), and inhibited the binding of E47 to DNA at equimolar concentrations. Size-exclusion chromatography, analytical ultracentrifugation and cross-linking experiments verified that this peptide prevented E47 dimerization. Furthermore, CD experiments provided evidence that helix II could induce a  $\beta$ -sheet secondary structure upon the highly  $\alpha$ -helical E47 bHLH domain.

**Conclusions:** This study is the first demonstration of dissociative inhibition in the bHLH class of transcription factors and also provides an example of  $\beta$ -sheet induction in an  $\alpha$ -helical protein. Future experiments will probe the structural determinants of the  $\beta$ -sheet secondary structure in helix II and investigate the generality of the dissociative strategy in other transcription factor families.

## Introduction

The design of agents to inhibit transcriptional activation of genes is an essential component of gene-targeted therapeutic strategies. One of the major methods developed for interfering with transcription has been the design of agents to interact directly with DNA [1]. Many classes of efficient DNA-binding agents have been developed, including intercalators [2], minor-groove binders [3,4], antisense [5] and triple-helix oligonucleotides [6], and carbohydrates [7]. Strategies designed to interfere directly with transcription factors have been approached with designed dominant-negative proteins, mimicking naturally occurring proteins that modulate transcription [8,9]. More recently, a new protein-protein dissociation based approach for regulating the biological functions of oligomeric proteins has been disclosed [10]. Thus, this strategy seems appropriate for application to the inhibition of dimeric transcription factors, such as the basic-helix-loop-helix protein (bHLH) E47.

E47 plays an essential role in activating expression of the immunoglobulin light-chain gene through binding at the  $\kappa$ E2 enhancer site [11]. The gene for E47 (E2A) is involved in chromosomal translocations that have been found in patients with pre-B-cell acute lymphoblastic leukemia [12]. E47 was one of the first transcription factors to be classified as a bHLH protein [13], and the determination of its

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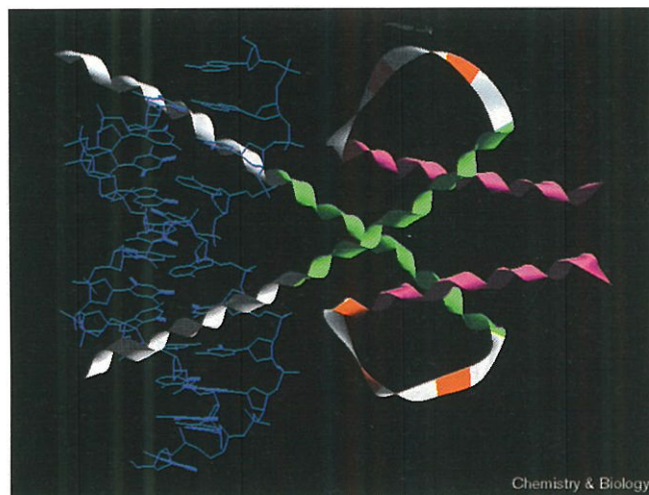
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co-crystal structure with DNA confirmed this prediction [14]. The structure of E47 consists of a highly basic region that binds into the major groove of DNA in a helical conformation, and two amphiphilic helices that form the four-helix bundle dimerization interface (Figure 1) [14,15].

The regulation of transcription by the bHLH proteins is governed by their ability to form homodimers or heterodimers [16]. Certain members of the HLH family of proteins can act as dominant-negative regulators of transcription, such as the protein Id [17]. The Id protein contains a HLH domain but lacks the basic region and preferentially binds with E47 or E12 proteins to form inactive complexes, thereby down-regulating bHLH-mediated transcription [18]. It was hypothesized that it would be possible to disrupt the dimerization of E47, and thereby prevent DNA binding, using interfacial peptides that correspond to the dimerization domain of E47. Thus, peptides corresponding to helix I and helix II (Figure 2) were explored as agents to inhibit the dimerization and DNA binding of this protein. We have found that these interfacial peptides were effective inhibitors of both dimerization and DNA binding as hypothesized. Furthermore, the most potent inhibitor, helix II, displayed  $\beta$ -sheet secondary structural features that we have correlated with its inhibitory potential.

Figure 1



Structure of E47 bound to DNA [14]. Helix I, green; helix II pink; the lysine residues available for cross-linking are red.

## Results and discussion

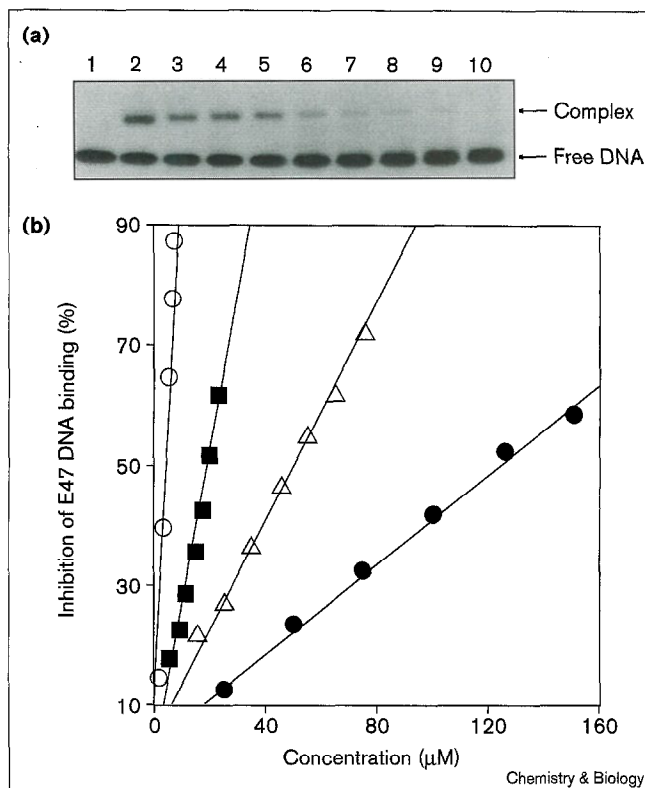
### Inhibition of E47 DNA binding by interfacial peptides

The inhibition of DNA binding by the interfacial peptides was monitored by gel mobility shift assays with E47 (8  $\mu$ M) and an oligonucleotide containing the  $\kappa$ E2 enhancer sequence (CAGGTG) [13,19] with added amounts of peptide (Figure 3). High concentrations of helix I were needed to inhibit E47 DNA binding ( $IC_{50}$  of 124  $\mu$ M; Figure 3b). Helix II, on the other hand, was a highly effective DNA-binding inhibitor (Figure 3a,b); at equimolar concentrations of helix II and E47 (8  $\mu$ M) DNA binding was abolished ( $IC_{50}$  of 3.7  $\mu$ M). Peptides corresponding to a truncated helix II, 2H10, and single point mutants, 2H10A4I (A4I, Ile4 $\rightarrow$ Ala), were also prepared to address the specificity of the peptide/protein interaction. 2H10 was less effective at blocking the DNA binding of E47 with an  $IC_{50}$  value of 19  $\mu$ M (Figure 3b), whereas 2H10A4I was even less effective with an  $IC_{50}$  value of 47  $\mu$ M (Figure 3b). These data suggest that a specific complex is forming between helix II and E47, as truncations of helix II, as in the 2H10 peptide, leads to a fivefold decrease in inhibition. Also, single point mutations, as in the 2H10A4I peptide, leads to a 12-fold decrease in inhibition relative to helix II.

Figure 2

	Basic	Helix I	Loop	Helix II
E47	ERRMANNAREVRVRDINEAFRELGRMCQMHLKSDKAQTKLLILQQAVQVILGLEQQVR			
Helix I		VRDINEAFRELGRMCQMHL		
Helix II				KLLILQQAVQVILGLEQQVR
2H10				KLLILQQAVQ
2H10A4I				KLLALQQAVQ

Figure 3



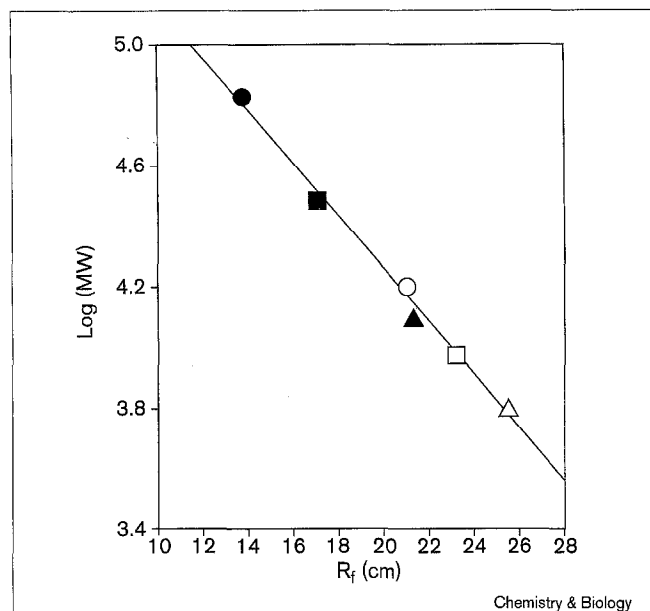
E47 DNA-binding inhibition assay. (a) A typical gel mobility shift assay for inhibition of E47 DNA binding with helix II: lane 1,  $^{32}$ P-labeled DNA alone (10 nM); lane 2, DNA and E47 (8  $\mu$ M); lane 3, DNA, E47 (8  $\mu$ M) and helix II (1  $\mu$ M); lane 4, DNA, E47 (8  $\mu$ M) and helix II (2  $\mu$ M); lane 5, DNA, E47 (8  $\mu$ M) and helix II (3  $\mu$ M); lane 6, DNA, E47 (8  $\mu$ M) and helix II (4  $\mu$ M); lane 7, DNA, E47 (8  $\mu$ M) and helix II (5  $\mu$ M); lane 8, DNA, E47 (8  $\mu$ M) and helix II (6  $\mu$ M); lane 9, DNA, E47 (8  $\mu$ M) and helix II (7  $\mu$ M); lane 10, DNA, E47 (8  $\mu$ M) and helix II (8  $\mu$ M). Buffer: 0.5 mM Tris-HCl, 0.1 mM EDTA, 0.1 mM DTT, 10% glycerol, pH 7.0, and 1  $\mu$ g of poly-dIdC per experiment. (b) Determination of  $IC_{50}$  values for helix I ●, helix II ○, 2H10 ■, and 2H10A4I △. The data for each curve were obtained from the densitometric analysis of the relevant autoradiograms for the inhibition of E47 DNA binding, and subsequently the linear portion of the sigmoidal curve was used for  $IC_{50}$  determination. There is an estimated  $\pm 10\%$  error in the  $IC_{50}$  values obtained.

### Probing dimerization inhibition

The disruption of E47 dimerization by the helix II peptide was investigated by size-exclusion chromatography, protein

The sequences of peptides derived from the helix-loop-helix domain of E47 that have been used in the course of this study.

Figure 4



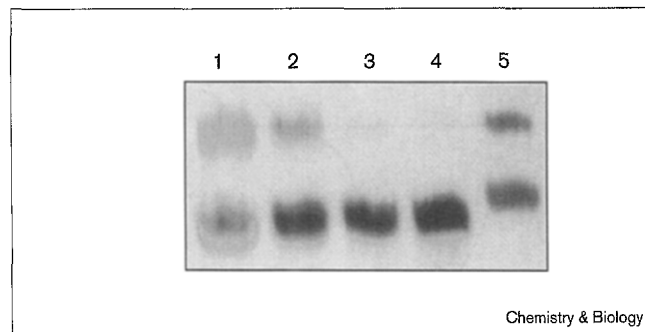
Size-exclusion chromatography. A standard molecular weight curve was generated using bovine serum albumin ●, carbonic anhydrase ■, cytochrome C ▲, and aprotinin △ and apparent molecular weights of E47 ○, and the complex of E47 and helix II □ were determined by interpolation.

cross-linking and analytical ultracentrifugation. The dimer of E47 eluted from a size-exclusion column as a single peak with an apparent molecular weight of 15,300 (Figure 4). When a mixture of E47 and helix II was applied to the same column, however, a single peak eluted corresponding to the molecular weight (MW) of the 1:1 complex (9,300, calculated 10,023), and no peak corresponding to the homodimer of E47 was detected (MW = 14,000).

E47 was also evaluated for inhibition of dimerization by cross-linking with sulfo-EGS (Pierce), and the monomer and cross-linked dimer were separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; Figure 5). Adding increasing amounts of helix II to E47 prior to the cross-linking reaction led to a decrease in the amount of cross-linked dimer formed, although a cross-linked peptide/protein band was not observed, presumably because of the lack of proximity between the single lysine residue in helix II with the three lysine residues in E47 (Figure 1). As a control, a lysine-containing peptide was evaluated for inhibition of dimeric E47 cross-linking, but no decrease in cross-linked protein was observed, even at a fivefold excess of the control peptide. These results also serve to indicate that a specific complex is formed between E47 and helix II.

Analytical equilibrium ultracentrifugation experiments were also performed to determine the aggregation state of

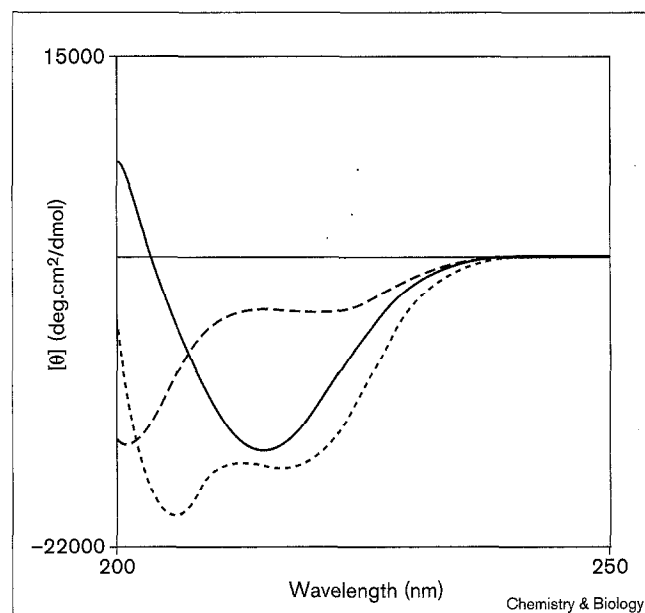
Figure 5



SDS-PAGE of cross-linking experiments with E47 and helix II: lane 1, E47 alone (10  $\mu$ M) and sulfo-EGS; lane 2, E47 (10  $\mu$ M), helix II (10  $\mu$ M), and sulfo-EGS; lane 3, E47 (10  $\mu$ M), helix II (20  $\mu$ M), and sulfo-EGS; lane 4, E47 (10  $\mu$ M). Buffer: 0.5 mM phosphate, 0.1 mM EDTA, 0.1 mM DTT, pH 7.0, and 5 mM sulfo-EGS per experiment.

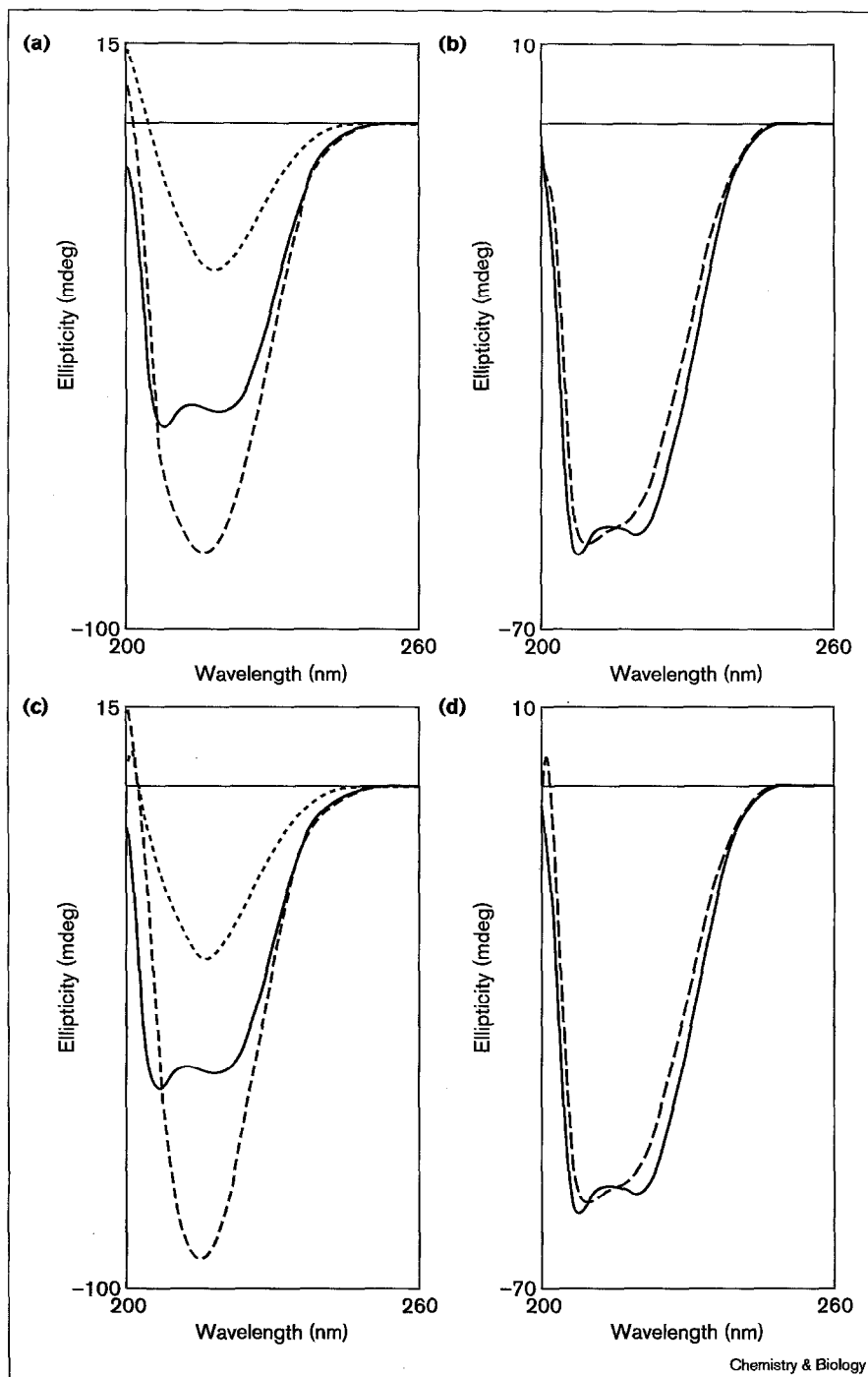
helix II, and to evaluate any changes in the dimerization of E47 in the presence of helix II (see the Supplementary material). Initial equilibrium experiments with helix II (100  $\mu$ M) established that the  $\beta$ -sheet peptide forms large aggregates composed of between 125 and 140 monomer units depending on the rotor speeds used (MWs of 293,000 and 331,000 at 3000 and 4000 rpm, respectively). E47 was found to sediment as a dimer at a rotor speed of 42,000 rpm ( $MW_{\text{calc'd}}$  13,810,  $MW_{\text{theor}}$  13,978), whereas a 1:1 mixture of E47 and helix II sedimented with a molecular weight of 8,330 at a rotor speed of 42,000 rpm (helix II is not

Figure 6



CD spectra of E47 (50  $\mu$ M; short dashes), helix I, (50  $\mu$ M; long dashes), and helix II (50  $\mu$ M; solid line). The buffer was composed of 0.5  $\mu$ M phosphate, 0.1 mM EDTA, 0.1 mM DTT, pH 7.0 at 25°C.

Figure 7



detectable at high rotor speeds). The molecular weight obtained for E47 with equimolar amounts of helix II is somewhat higher than what one would anticipate for a monomer of E47 (MW 6989), and slightly lower than the anticipated molecular weight of a 1:1 complex between E47 and helix II (MW 9320). Although these results do not unequivocally support the possible formation of a 1:1 complex between E47 and helix II, they do leave open the

possibility that such a complex exists, as indicated by size-exclusion chromatography and DNA-binding experiments. These data do support the role of helix II in disrupting the dimerization of E47 at equimolar concentrations.

#### Secondary structural evaluation of interfacial peptides

Circular dichroism (CD) was performed to investigate the structural basis of inhibition by the helix I and helix II

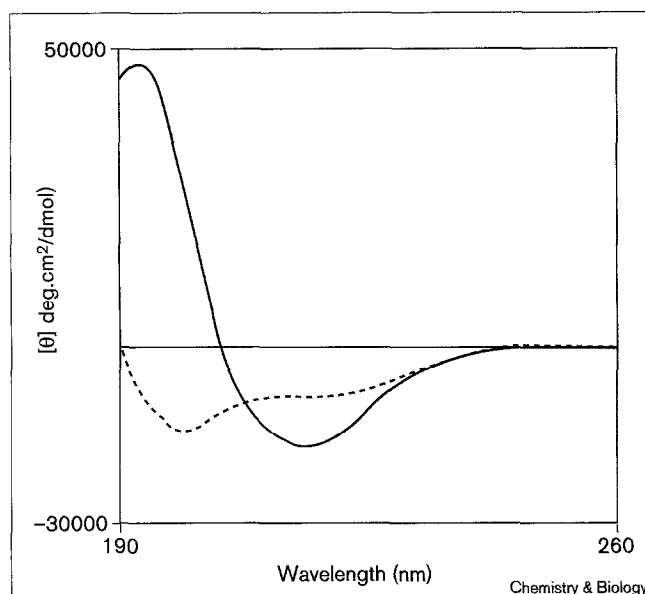
peptides. The CD spectrum of E47 contained two minima at 208 and 222 nm (Figure 6) as is observed for helical proteins [20]. Surprisingly, the CD spectrum of helix II corresponded to the spectrum that one would obtain from a  $\beta$ -sheet-containing protein (Figure 6), in that a single minimum was observed at 218 nm, whereas helix I displayed CD spectra characteristic of a random coil [20].

In order to examine any change in the secondary structure of E47 in the presence of helix II (the most potent inhibitor), CD spectra were acquired for a mixture of helix II (50 mM) and E47 (50 mM; Figure 7a) and also helix II (75 mM) and E47 (50 mM) (Figure 7c). The spectra of the complexes (E47–helix II) displayed a significantly larger peak at 218 nm than that obtained for E47 alone (Figure 7a,c). The change in the secondary structure of E47 was evidenced by subtracting the spectra of the 50 mM and 75 mM solution of helix II from the spectra of their respective complexes (Figure 7b,d). The difference spectra of E47 thus obtained showed a significant shift of the minima from 222 nm (indicative of  $\alpha$  helix) to 218 nm (indicative of  $\beta$  sheet), especially with an 1.5 times excess of added helix II (Figure 7d). This structural transition in E47 may imply that helix II is inducing a  $\beta$ -sheet character in E47.

Chou–Fasman secondary structural predictions [21] indicated that the first 16 residues in the carboxy-terminal helix of E47, KLLILQQAVQVILGLE (using single-letter amino acid code), have the potential to adopt  $\beta$ -sheet structure. Thus the secondary structure of the peptides 2H10, and 2H10A4I were also investigated using CD spectroscopy (Figure 8). It was shown that the peptide 2H10 was capable of adopting a  $\beta$ -sheet structure, whereas 2H10A4I adopted a random-coil structure. This is perhaps not surprising, given the importance of  $\beta$ -branched isoleucine residues in promoting  $\beta$ -sheet secondary structure, in both statistical [22] and experimental studies [23,24]. It is remarkable, however, that a single residue Ile4 $\rightarrow$ Ala mutation, not only abolishes the  $\beta$ -sheet structure but substantially diminishes the inhibitory potential (Figure 3b). This adds further credence to the importance of the  $\beta$ -sheet structure in modulating the inhibitory properties of these peptides.

The results from CD spectroscopy provide a possible mechanism of inhibition for E47–DNA complexation. In this mechanism, helix II, a peptide that exists in a helical conformation when in the protein framework of E47, adopts a  $\beta$ -sheet structure in solution and induces a corresponding structural change in E47, thereby abrogating DNA binding. Furthermore, evidence from size-exclusion chromatography, cross-linking and analytical ultracentrifugation studies support the role of the 20 amino acid peptide helix II in inhibiting the dimerization and DNA binding of the 59 amino acid protein E47. The induction of

**Figure 8**



CD spectra of 2H10 (50  $\mu$ M; solid line) and 2H10A4I (50  $\mu$ M; dashed line). The buffer was composed of 0.5 mM phosphate, 0.1 mM EDTA, 0.1 mM DTT, pH 7.0 at 25°C.

$\beta$ -sheet structure in a helical protein is not unprecedented [25]; the progression of the prion disease state is believed to occur by such a mechanism [26]. Cohen and coworkers [27,28] have shown that a prion-derived peptide was able to induce  $\beta$ -sheet structure in other prion-derived peptides that have random-coil or  $\alpha$ -helical secondary structures. Furthermore, recent work by Minor and Kim [29] and Regan and coworkers [30] has delineated the importance of tertiary interactions as determinants for secondary structure. Thus helix II provides a new example of an inherently  $\beta$ -sheet forming sequence that can exist as an  $\alpha$  helix in the protein framework of E47.

The use of interfacial peptides for the inhibition of oligomeric enzymes as well as receptor–protein-mediated signaling has received considerable attention recently [10]. The application of this protein–protein disruption strategy has not been extended to the realm of dimeric transcription factors, however. Thus, the development and implementation of peptide-based dimerization inhibitors in this study might prove to be of considerable use in the direct and specific targeting of transcription factors that are implicated in abnormal cellular proliferation.

### Significance

The inhibition of transcription factors is a potential therapeutic strategy and requires the design of suitable inhibitory agents. This study demonstrates the inhibition of the basic helix–loop–helix (bHLH) transcription factor E47 using peptides corresponding to its dimerization

interface. The identification of helix II, a  $\beta$ -sheet-forming peptide that abrogates E47 dimerization and DNA binding at equimolar concentrations, underscores the potential for the dimerization inhibition strategy. Moreover, the induction of  $\beta$ -sheet structure upon the predominantly  $\alpha$ -helical E47 protein by helix II highlights the structural plasticity of the E47 tertiary structure. This study therefore not only provides a rational framework for the design of dimerization inhibitors, but also opens the door for the conformational analysis of peptides derived from the bHLH family of transcription factors, which might help delineate the factors that govern the secondary and tertiary structures of this class of proteins.

## Materials and methods

### Synthesis of peptides

The peptides: helix I, helix II, 2H10, and 2H10A4I, were synthesized using solid phase methodology on a Rink amide resin [31] using a fluorenylmethyloxy-carbonyl (Fmoc)-based strategy [32] on a Biosearch 9500 peptide synthesizer. The synthesis and characterization of E47 together with the general synthesis and cleavage procedures for resin-bound peptides have been previously described [19]. All peptides were purified to homogeneity using high-performance liquid chromatography (HPLC) on a Vydac C8 reverse phase semi-preparative column, 2.2  $\times$  25 cm, 8.0 ml/min, mobile phase A (100%  $\text{CH}_3\text{CN}$ , 0.1% TFA) and mobile phase B (100%  $\text{H}_2\text{O}$ , 0.1% TFA). Peptides were purified with linear gradients (10–50% for helix I; 15–55% for helix II; 10–65% for 2H10 and 2H10A4I) over 60 min on a Waters Delta Prep 4000. The eluant was monitored at 214 nm. The peptides were judged to be greater than 95% pure by analytical reverse-phase HPLC. The peptides were characterized by plasma-desorption mass spectrometry, and amino-acid analysis (see the Supplementary material).

### Electrophoretic mobility shift assays

EMSAs were performed with  $^{32}\text{P}$ -labeled 29 base pair double stranded oligonucleotide probe containing a CAGGTG DNA-binding sequence [33]. The 5' sense strand of the DNA probe is as follows: 5'-AATTCGCGGCCGCGAGAACGGTGGTCCAG-3'. The DNA was radiolabeled using the Klenow fragment of DNA Polymerase (New England Biolabs) in the presence of dCTP, dGTP, dTTP and 5'- $\alpha$ - $^{32}\text{P}$ -dATP (Amersham) and purified through a Nick column (Pharmacia-Biotech) containing Sephadex G-25. Typical inhibition assays involved the incubation of E47 (10 mM) with or without a given concentration of the peptide inhibitor for 1 hour in Buffer A (0.5 mM phosphate, 0.1 mM EDTA, 0.1 mM DTT, at pH 7.0). This was followed by the addition of 10 nM labeled DNA probe, 10% glycerol and 1 mg poly-dIdC and further incubation for 30 min in a total volume of 20  $\mu\text{l}$  at room temperature. The reactions were analyzed by 8% nondenaturing PAGE. Densitometric analysis of the relevant autoradiograms allowed the measurement of the relative intensities of the protein/DNA bands. Sigmoidal curves were obtained by plotting the decrease in intensity of the protein/DNA band as a function of inhibitor concentration. The  $\text{IC}_{50}$  values were determined by a least squares analysis of the linear portion of the sigmoidal curves.

### Size-exclusion chromatography

A solution of E47 (250  $\mu\text{l}$ , 200  $\mu\text{M}$ ) and helix II (250  $\mu\text{l}$ , 800 mM) was run through Sephadex G-50 (90  $\times$  1.6 cm column) with a flow rate of 0.3 ml/min at 4°C, and a buffer composed of 10 mM phosphate, 50 mM NaCl, buffer at pH 7.0. The eluant was monitored at 214 nm. A standard molecular weight curve was generated using bovine serum albumin, carbonic anhydrase, cytochrome C, and aprotinin, and apparent molecular weights were determined by interpolation.

### Cross-linking experiments

Cross-linking experiments were performed at room temperature in Buffer A with a 500-fold excess of an amine-reactive cross-linking reagent,

sulfo-EGS (Pierce). E47 was incubated in the presence or absence of an appropriate concentration of helix II for 2 h, upon which the reaction was stopped by the addition of gel-loading buffer and subsequent heating to 100°C. The reactions were analyzed immediately by 17% SDS-PAGE and the resulting protein bands were visualized by silver staining. A control cross-linking experiment was also performed with E47 in the presence of a peptide containing six lysine residues, EELEKKLKEEEK-LKK-NH<sub>2</sub> [34]. The concentrations of E47, helix II, and the control peptide were verified by amino-acid analysis.

### Analytical ultracentrifugation

Analytical equilibrium ultracentrifugation was carried out on a Beckman XLA analytical ultracentrifuge (Purdue Cancer Center) at 25°C with peptide samples in 0.5 mM phosphate buffer at pH 7.0. The partial specific volumes were calculated on the basis of the amino acid composition. Equilibrium data were collected over a period of 48 h in all cases and equilibrium was judged complete when successive scans were superimposable. The absorbance was monitored at 214 nm for helixII, and 230 nm for E47 and the 1:1 mixture of E47 and helix II. The data were subjected to global analysis with the program SEDEQ and fit to a single molecular weight species for ease of interpretation [35].

### Circular dichroism spectroscopy

CD spectra were recorded on a Jasco J600 spectropolarimeter at 24°C in Buffer A using a 0.2 cm path length cell. CD spectra for E47, helix I, helix II, 2H10, 2H10A4I were recorded at 50  $\mu\text{M}$  concentrations. For experiments to determine the change in secondary structure of E47 in the presence of helix II, E47 was incubated for 120 min with or without helix II for 1 h in Buffer A at room temperature. Subsequently, the CD spectra of (i) helix II (50  $\mu\text{M}$ ), (ii) E47 (50  $\mu\text{M}$ ), (iii) E47 (50  $\mu\text{M}$ ) and helix II (50  $\mu\text{M}$ ) were recorded as an average of four scans from 260 to 200 nm with a resolution of 0.2 nm and a scan rate of 10 nm/min (Figure 7a). The spectra of the E47 alone, (ii), was subtracted from the spectra of E47 and helix II, (iii), to give a spectra of E47 in the presence of helix II (Figure 7b). The spectra for E47 in the presence of 75  $\mu\text{M}$  helix II was treated similarly (Figure 7c,d). The concentration of E47 and helix II was determined from amino-acid analysis. The assumption was made that the secondary structure of helix II is not prone to change as was evidenced by thermal and Urea denaturation experiments (see the Supplementary material).

### Supplementary material available

Characterization of the peptides used in this study, analytical ultracentrifugation data and thermal and urea denaturation studies with helix II using circular dichroism are available upon request and on the internet.

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## References

1. Broggin, M. & D'Incalci, M. (1994). Modulation of transcription factor-DNA interactions by anticancer drugs. *Anti-Cancer Drug Design* **9**, 373–387.
2. Baguley, B.C. (1991). DNA intercalating antitumor agents. *Anti-Cancer Drug Design* **6**, 1–35.
3. Pindur, U. & Fischer, G. (1996). DNA complexing minor groove-binding ligands: antitumor and antimicrobial drug design. *Curr. Med. Chem.* **3**, 379–406.
4. White, S., Szewczyk, J.W., Turner, J.M., Baird, E.E. & Dervan, P.B. (1998). Recognition of the four Watson-Crick base pairs in the DNA minor groove by synthetic ligands. *Nature* **391**, 468–471.
5. Gewirtz, A.M. (1997). Antisense oligonucleotide therapeutics for human leukemia. *Crit. Rev. Oncogenesis* **8**, 93–109.
6. Maher, J.L. (1992). DNA triple-helix formation—an approach to artificial gene repressors. *BioEssays* **14**, 807–815.

7. Hunziker, J. (1996). Nucleic acid ligands based on carbohydrates. *Chimia* **391**, 468-471.
8. Krylov, D., Kasai, K., Echlin, D.R., Taparowsky, E.J., Arnheiter, H. & Vinson, C. (1997). A general method to design dominant negatives to B-HLHZip proteins that abolish DNA binding. *Proc. Natl Acad. Sci. USA* **94**, 12274-12279.
9. Herskowitz, I. (1987). Functional inactivation of genes by dominant negative mutations. *Nature* **329**, 219-222.
10. Zutshi, R., Brickner, M., & Chmielewski, J. (1998). Inhibiting the assembly of protein-protein interfaces. *Curr. Opin. Chem. Biol.* **2**, 62-66.
11. Lenardo, M., Pierce, J.W., & Baltimore, D. (1987). Protein-binding sites in Ig gene enhancers determine transcriptional activity and inducibility. *Science* **236**, 1573-1577.
12. Look, A.T. (1997). Oncogenic transcription factors in human acute leukemias. *Science* **278**, 1059-1064.
13. Murre, C., McCaw, P.S., & Baltimore, D. (1989). A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD, and myc proteins. *Cell* **56**, 777-783.
14. Ellenberger, T., Fass, D., Arnaud, M., & Harrison, S.C. (1994). Crystal structure of transcription factor E47: E-box recognition by a basic region helix-loop-helix dimer. *Genes Dev.* **8**, 970-980.
15. Anthony-Cahill, S.J., et al., & DeGrado, W.F. (1992). Molecular characterization of helix-loop-helix peptides. *Science* **255**, 979-983.
16. Murre, C., et al., & Stuver, M.H. (1994). Structure and function of helix-loop-helix proteins. *Biochim. Biophys. Acta.* **1218**, 129-135.
17. Benzeza, R., Davis, R.L., Lokshon, D., Turner, D.L. & Weintraub, H. (1990). The protein Id: a negative regulator of helix-loop-helix DNA binding proteins. *Cell* **61**, 49-59.
18. Langlands, K., Yin, X., Anand, G. & Prochownik, E.V. (1997). Differential Interactions of Id proteins with basic-helix-loop-helix transcription factors. *J. Biol. Chem.* **272**, 19785-19793.
19. Bishop, P., Jones, C., Ghosh, I. & Chmielewski, J. (1995). Synthesis of the basic-helix-loop-helix region of the immunoglobulin enhancer binding protein E47 and evaluation of its structural and DNA binding properties. *Int. J. Pept. Protein Res.*, **46**, 149-154.
20. Greenfield, N. & Fasman, G.D. (1969). Computed circular dichroism spectra for the evaluation of protein conformation. *Biochemistry* **8**, 4108-4116.
21. Chou, P.Y., & Fasman, G.D. (1974). Prediction of protein conformation. *Biochemistry* **13**, 222-245.
22. Chou, P.Y., & Fasman, G.D. (1974). Conformational parameters for amino acids in helical,  $\beta$ -sheet, and random coil regions calculated from proteins. *Biochemistry* **13**, 211-222.
23. Smith, C.K., Withka, J.M., & Regan, L.A. (1994). Thermodynamic scale for the beta-sheet forming tendencies of the amino-acids. *Biochemistry* **33**, 5510-5517.
24. Minor, D.L., & Kim, P.S. (1994). Context is a major determinant of beta-sheet propensity. *Nature* **371**, 264-267.
25. Mihara, H. & Takahashi, Y. (1997). Engineering peptides and proteins that undergo alpha-to-beta transitions. *Curr. Opin. Struct. Biol.* **7**, 501-508.
26. Ng, S. B. L., & Doig, A. J. (1997). Molecular and chemical basis of prion-related diseases. *Chem. Soc. Rev.* **26**, 425-432.
27. Pan, K.-M., et al., & Prusiner, S.B. (1993). Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. *Proc. Natl Acad. Sci. USA* **90**, 10962-10966.
28. Nguyen, J., Baldwin, M.A., Cohen, F.E., & Prusiner S.B. (1995). Prion protein-peptides induce alpha-helix to beta-sheet conformational transitions. *Biochemistry* **34**, 4186-4192.
29. Minor, D.L. & Kim, P.S. (1996). Context-dependent secondary structure formation of a designed protein sequence. *Nature* **380**, 730-734.
30. Dalal, S., Balasubramanian, S. & Regan, L. (1997). Protein alchemy: Changing beta-sheet into alpha-helix. *Nat. Struct. Biol.* **4**, 548-552.
31. Rink, H., (1987). Solid-phase synthesis of protected peptide fragments using a trialkoxydiphenyl-methylester resin. *Tetrahedron Lett.* **28**, 3787-3790.
32. Atherton, E. & Sheppard, R.C. (1987). In *The Peptides*. (Gross, E. & Meienhofer, J. eds) Vol. 9, pp 1-38. Academic Press, New York.
33. Bishop, P., Ghosh, I., Jones, C. & Chmielewski, J. (1995). Basic-helix-loop-helix region of Tal: evaluation of structure and DNA affinity. *J. Am. Chem. Soc.* **117**, 8283-8284.
34. Luttring, R.A., Lipton, M. & Chmielewski, J. (1996). Differential self assembly of amphiphilic helical peptides. *Amino Acids* **10**, 295-304.
35. Shuster, T.M. & Laue, T.M. (1994). *Modern Analytical Ultracentrifugation*. Birkhauser, Boston.

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